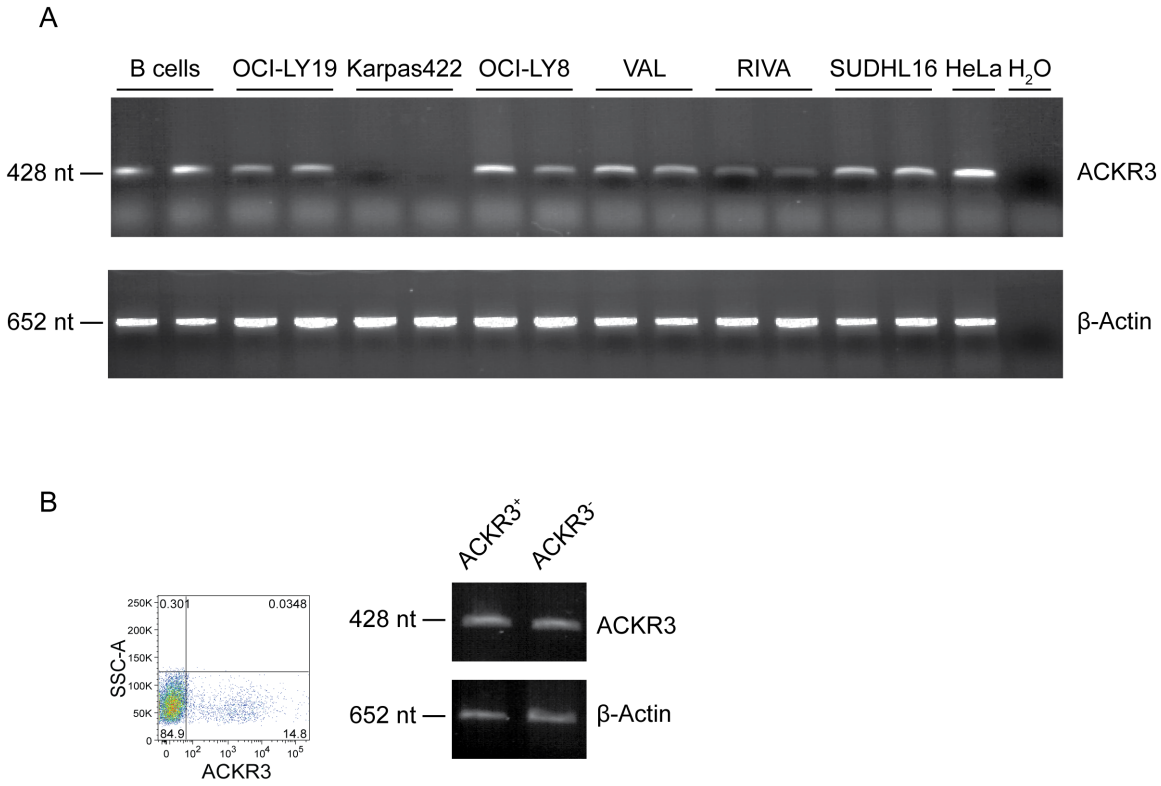
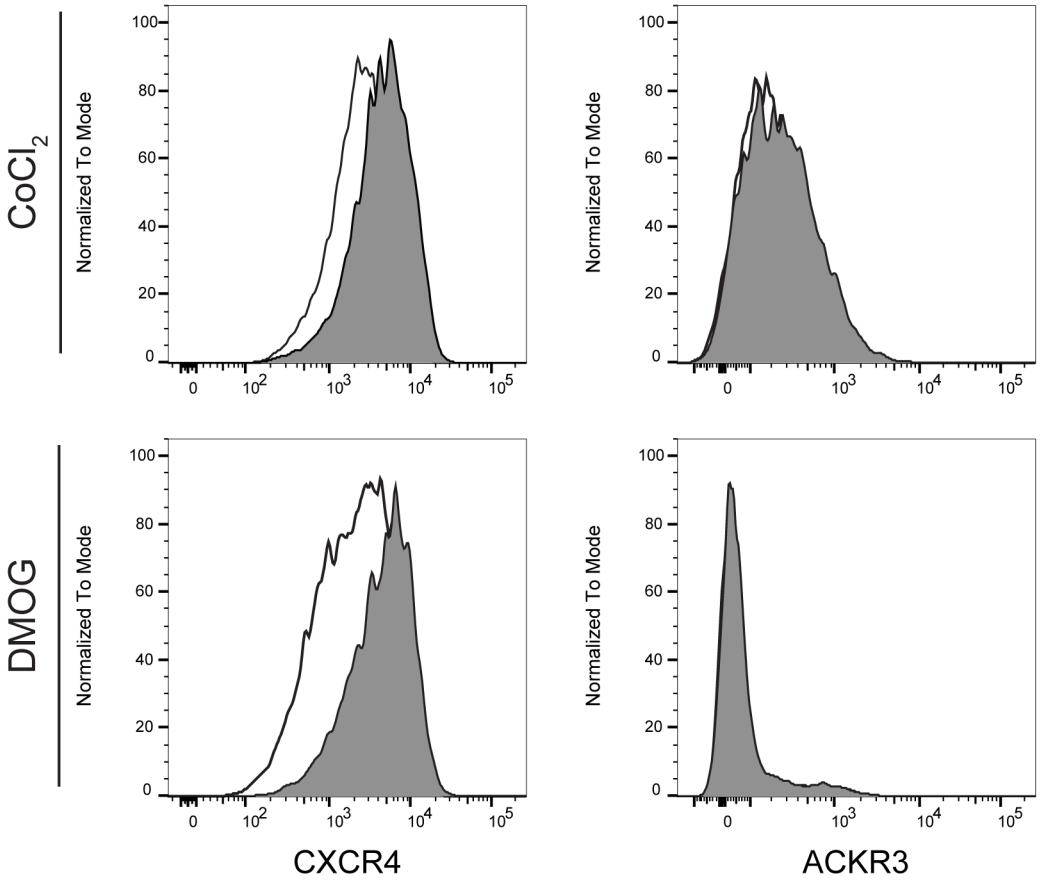
ACKR3 expression on diffuse large B cell lymphoma is required for tumor spreading and tissue infiltration

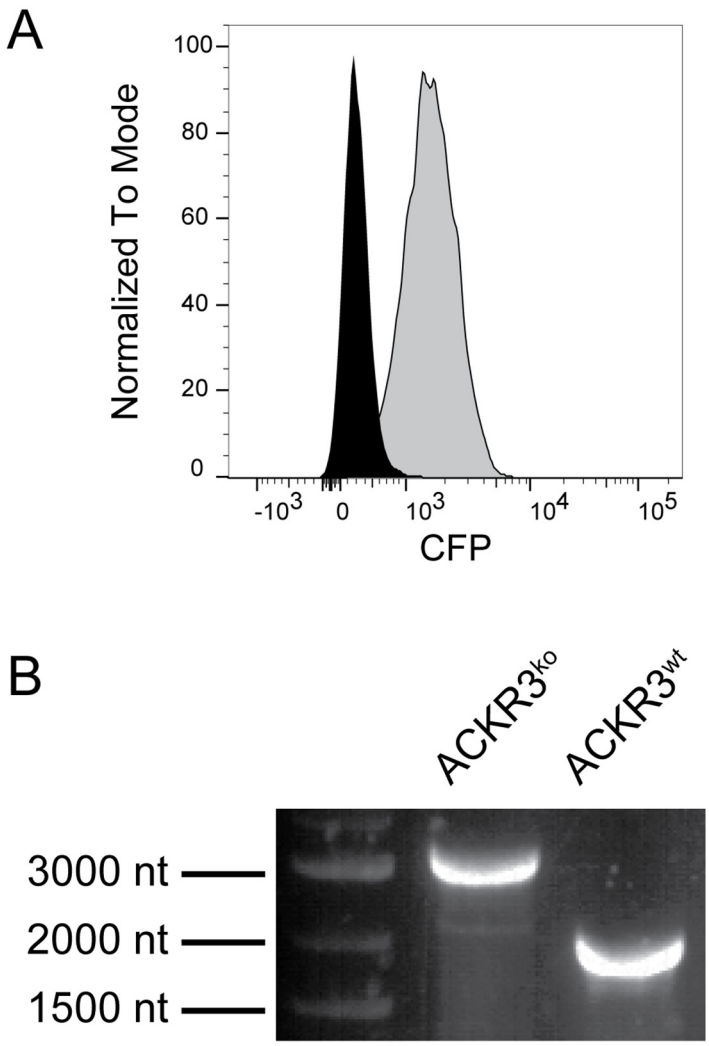
SUPPLEMENTARY MATERIALS



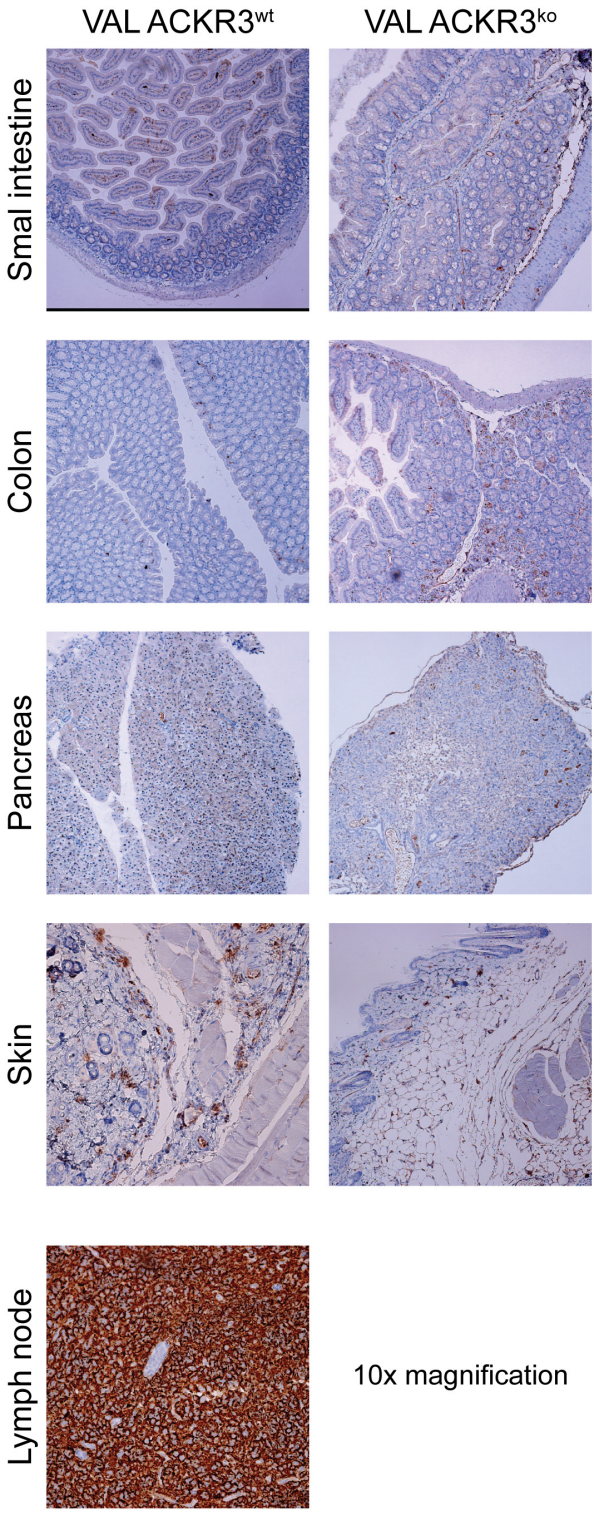
Supplementary Figure 1: (A) ACKR3 is expressed in selected GCB-DLBCL cell lines. Semi-quantitative PCR of ACKR3 transcripts is shown. ACKR3 is expressed on OCI-LY19, OCI-LY8, VAL, RIVA and SUDHL16 cell lines, but not on Karpas422 cells. Peripheral blood human B cells and HeLa cells were used as positive controls. β-Actin mRNA was measured as internal control. Representative data of one of at least three independent experiments. **(B)** Regulation of ACKR3 surface expression is independent from *ACKR3* gene transcription levels.ACKR3 surface expression was measured by flow cytometry using mAb 11G8. Cells were sorted for ACKR3 expression. $ACKR3^{+}$ and $ACKR3^{-}$ subpopulations of VAL cells (left panel) express similar mRNA levels (right panel) as revealed by semi quantitative PCR.



Supplementary Figure 2: CXCR4 (left) but not ACKR3 (right) is upregulated in response to hypoxia. VAL cells were cultured in the absence (open histograms) or in the presence of the hypoxia-miming compounds $CoCl_2$ (100 μM) or DMOG (500 μM) (grey histograms). Surface expression of ACKR3 (mAb 9C4) and CXCR4 (mAB 12G5) were evaluated by flow cytometry. Representative plots of three independent experiments.



Supplementary Figure 3: (A) Expression of CFP in ACKR3^{ko} cells (gray) analyzed by flow cytometry. ACKR3^{wt} cells (black) were used as control. **(B)** PCR of genomic DNA from ACKR3^{ko} and ACKR3^{wt} cells. PCR analysis of ACKR3 genomic DNA in wild type and CRISPR/Cas9 treated VAL cells. Primers were designed in order to cover the 5' genomic DNA region up to the 3' outside regions flanking of *ACKR3* gene, so that the predicted lengths of amplified fragments are 3312 nt in case of insertion of the HDR construct (ACKR3^{ko} cells) and 2128 nt in the original.



Supplementary Figure 4: Immunohistochemistry of tissue invasion by VAL ACKR3^{ko} and VAL ACKR3^{wt} cells. NOD/SCID/common γ-chain^{ko} mice were injected with 2x10^{5} cells. Organs were removed after four weeks, formalin-fixed, paraffin-embedded and sections stained for CD20 (brown) expressing human VAL cells and counterstained with hematoxylin (blue).